

ABSTRACT OF THE DISCLOSURE

In one embodiment of the invention, a method is provided for preparing plasmid from host cells which contain the plasmid, comprising: (a) providing a plasmid solution comprised of unligatable open circular plasmid; (b) reacting the unligatable open circular plasmid with one or
5 more enzymes and appropriate nucleotide cofactors, such that unligatable open circular plasmid is converted to 3'-hydroxyl, 5'-phosphate nicked plasmid; (c) reacting the 3'-hydroxyl, 5'-phosphate nicked plasmid with a DNA ligase and DNA ligase nucleotide cofactor, such that 3'-hydroxyl, 5'-phosphate nicked plasmid is converted to relaxed covalently closed circular plasmid; and (d) reacting the relaxed covalently closed circular plasmid with a DNA gyrase and
10 DNA gyrase nucleotide cofactor, such that relaxed covalently closed circular plasmid is converted to negatively supercoiled plasmid. In other embodiments, DNA gyrase is replaced by reverse DNA gyrase or reaction (d) is not performed.